SCIENTIFIC REPORTS

OPEN

First Biosynthetic pathway of 1-hepten-3-one in *Iporangaia pustulosa* (Opiliones)

Daniele F. O. Rocha[1], Felipe C. Wouters[1], Glauco Machado[2] & Anita J. Marsaioli[1]

[1]Chemistry Institute, State University of Campinas, POB 6154, 13083-970 Campinas, SP, Brazil, [2]Departamento de Ecologia, Instituto de Biociências, Universidade de São Paulo, Rua do Matão, trav.14, no. 321, 05508-090 São Paulo, SP, Brazil.

Arthropods produce a great variety of natural compounds, many of which have unexplored biosynthesis. Among the armored harvestmen (Arachnida: Opiliones) of the suborder Laniatores, the defensive gland exudates contain vinyl ketones and other constituents of supposed polyketide origin. We have studied the biosynthesis of 1-hepten-3-one in the Neotropical harvestman *Iporangaia pustulosa* by feeding individuals with ^{13}C-labeled precursors, demonstrating its mixed acetate/propionate origin. ^{13}C NMR spectroscopy showed an unusual labeling pattern suggesting different propionate sources for starting and extender units. Our analysis also indicates the presence of methylmalonyl-CoA mutase, converting acetate into propionyl-CoA via succinyl-CoA, together with other C_3 unit routes. This is the first biosynthetic study of alkyl vinyl ketones in arthropods. Our results shed light on the origin and diversification of chemical compounds in a major arthropod group.

Arthropods produce a great variety of natural compounds that are used in inter- and intra-species chemical communication. Although the identity of these info-chemicals have been intensively explored over the last 60 years[1], their biosynthesis remains vastly unexplored when compared to plants and microorganisms[2]. Moreover, most biosynthetic studies with arthropods focus on insects, and only a few on other major arthropod groups such as arachnids, whose species may produce venoms, repellent exudates, and pheromones[1]. Arachnids of the order Opiliones, for instance, have a pair of exocrine glands that release a great variety of odorous compounds[3] (Fig. 1) used as alarm pheromones[4] and defense against predators[5–7]. However, their biosynthetic pathways have never been investigated.

Among the armored harvestmen of the suborder Laniatores, the gland exudates contain mixtures of benzoquinones, alkylated phenols, or alkyl vinyl ketones[8,9]. Considering the phylogeny of the Neotropical family Gonyleptidae, in particular, the production of benzoquinones is the ancestral state, with at least four independent losses of these compounds[10]. The production of vinyl ketones, on the other hand, is derived and represents a synapomorphy of a major clade composed of five subfamilies and the production of alkyl-phenols is also derived and evolved at least five times independently[10]. The biosynthetic pathways of these gland exudate constituents in harvestmen may bring information on the putative origins and diversification of chemical compounds within this major arachnid group.

In a previous report, we detected 1-hepten-3-one (**1**) as the major component in the scent gland exudates of the gonyleptid harvestman *Iporangaia pustulosa*[9]. Similar vinyl ketones were also found in other gonyleptid harvestmen belonging to the clade known as K92[8,10]. Here we describe the biosynthetic pathway of **1**, revealing the condensation of Pr + Ac + Pr units to produce a polyketide chain. This pathway depends on a methylmalonyl-CoA mutase and other enzymes comprising an ensemble of parallel propionate/methylmalonate metabolic routes. Further, we revealed that there are different sources of C_3 depending on the unit role of starter or extender. To our knowledge, this is the first biosynthetic study of an alkyl vinyl ketone in arthropods.

Results

Feeding and analysis. Four ^{13}C-labeled precursors, denoted [$^{13}C_3$]propionate, [4-^{13}C]methylmalonate, [1-^{13}C] acetate and [1-^{13}C]glucose, were added to the diet of *Iporangaia* individuals (males and females) and their incorporation into **1** was monitored by ^{13}C NMR spectroscopy. The vinyl ketone **1** produced by *Iporangaia* individuals in each feeding experiment showed specific incorporation of the precursors.

Figure 1 | Female of the harvestman *Iporangaia pustulosa* releasing two white droplets of scent gland exudate in the lateral margins of the carapace. The photo was taken by Prof. Glauco Machado.

[¹³C₃]propionate incorporation. The enrichment by [¹³C₃]propionate is given by the integration ratio of the satellite lines by the sum of all carbon signals[11,12] (**Fig. 2, Table 1**). Positions C-1, C-2, C-5, C-6 and C-7 were labeled, and at C-1 and C-2 the enrichment was weaker. All labeled positions showed increased satellite lines.

[4-¹³C]methylmalonate, [1-¹³C]acetate and [1-¹³C]glucose incorporation. For each carbon signal of **1** in the ¹³C NMR spectrum, the ratio of the signal height was calculated using one non-enriched position (C-4 for [1-¹³C]acetate and [4-¹³C]methylmalonate; C-5 for [1-¹³C]glucose), furnishing the R value. The $r = R_{labeled\ group}/R_{control\ group}$ was calculated for all the positions of **1**. The enrichment at each site was obtained by multiplying r by 1.1, which is the natural abundance of ¹³C (**Table 2**)[11,12]. Experiment with [4-¹³C]methylmalonate produced **1** enriched at C-1 (1.3%), C-6 (1.4%) and C-7 (1.5%) (Supplementary Data S3). Individuals fed on [1-¹³C]acetate produced **1** showing increased intensities at C-1 (2.5%), C-3 (3.0%), C-5 (1.6%) and C-6 (1.3%) (Supplementary Data S4). [1-¹³C]glucose incorporation yielded **1** with isotopic enhancement

Table 1 | Enrichment of 1-hepten-3-one (1) from the harvestman *Iporangaia pustulosa* after [¹³C₃]propionate incorporation

C	% ¹³C-¹³Cᵃ	J c-c (Hz)	Position
1	15.25	66.85	2
2	16.17	66.87	1
3		Not enriched	
4		Not enriched	
5	39.18	27.51	6
6	48.63	34.73	7
		32.39	5
7	45.06	34.55	6

at C-2, C-4, C-6 and C-7 of 1.3% (Supplementary Data S5), with a lower global incorporation and ¹³C NMR signal enhancement than observed in [1-¹³C]acetate feeding experiment. The sample was too diluted and the carbonyl signal was not observed.

Discussion

Given that the biosynthesis of secondary metabolites of harvestmen had never been studied before, we used Morgan's suggestion[2] of an aceto-propiogenin origin for aliphatic ketones structurally similar to **1** as a first approach. To test this hypothesis, we selected ¹³C NMR spectroscopy and ¹³C-labeled precursors (acetate and propionate), which allowed the exact position of the unit incorporation into the intact molecule of **1**. *Iporangaia pustulosa* has a defense secretion with **1** as major constituent, allowing ¹³C NMR analysis of the crude exudate, with the additional advantage of surviving feeding experiments in the laboratory.

Feeding *Iporangaia* individuals on [¹³C₃]propionate yielded **1** labeled at C-1, C-2, C-5, C-6 and C-7 (Table 1). The ¹³C enrichment increased the intensities of the satellites and reduced the central signal of the singly labeled isotopomers. The doublets at C-7 and C-5 and double doublet at C-6 have characteristic ¹³C-¹³C coupling constants, revealing the intact incorporation of the [¹³C₃]propionate precursor at these positions (Fig. 2, Table 2). Satellite signals (doublets) were also enhanced at C-1 and C-2 indicating the incorporation of a second [¹³C₃]propionate unit and loss of one labeled carbon. The ¹³C enrichment at C-1 and C-2 (~15%) was less effective than at C-5, C-6 and C-7 (~44%), suggesting alternative C₃ units for initiating (propionate) and extending (methylmalonate) the polyketide chain
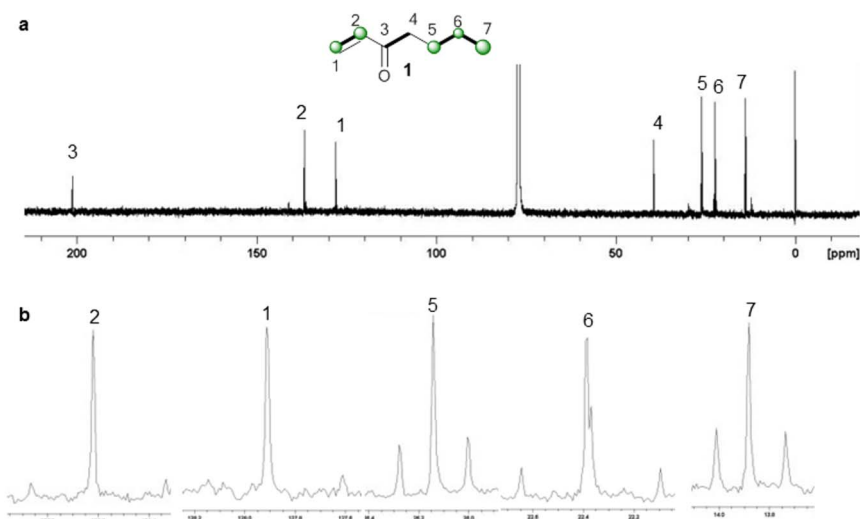

Figure 2 | Results of the experiment in which *Iporangaia pustulosa* individuals were fed [¹³C₃]propionate. (a) ¹³C NMR spectrum (125 MHz, CDCl₃) of the exudate containing **1** as major compound. (b) Extended signals of ¹³C enriched positions showing ¹³C-¹³C coupling patterns.

Table 2 | Labeling and ^{13}C enrichment of 1-hepten-3-one (1) from feeding of *Iporangaia pustulosa* individuals with labeled precursors

		[1-^{13}C] acetate	[4-^{13}C] methyl-malonate	[1-^{13}C] glucose
C	δ (ppm)	% ^{13}C	% ^{13}C	% ^{13}C
1	127.9	**2.5**	**1.3**	Not measured[a]
2	136.6	0.9	0.4	**1.3**
3	201.2	**3.0**	0.7	Not measured[b]
4	39.4	1.1	1.1	**1.3**
5	26.1	**1.6**	1.1	1.1
6	22.8	**1.3**	**1.4**	**1.3**
7	13.9	0.9	**1.5**	**1.3**

Signals in bold type represent significant incorporation in groups 1, 2 and 3.
[a]C-1 signal was overlapped by the solvent signal.
[b]C-3 signal not visualized due to sample dilution and it was considered not enriched.
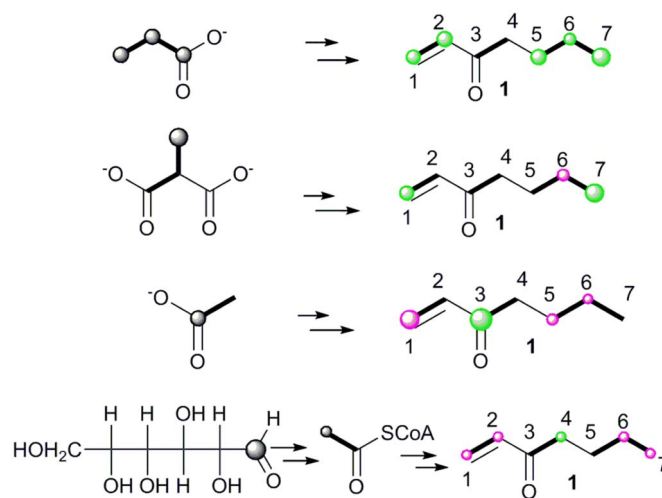

Figure 4 | Labeling pattern of 1 by feeding *Iporangaia pustulosa* individuals with ^{13}C labeled precursors. Colored ball indicate ^{13}C labeled positions. Green signals means expected and magenta means unexpected labeling. The ball sizes correspond to ^{13}C enrichment (%).

of **1**. In the same way, [4-^{13}C]methylmalonate incorporation yielded labelling at C-1 and C-7, in agreement with two C_3 units incorporated into the polyketide chain of **1**. C-3 and C-4 showed no ^{13}C incorporation from C_3 labeled precursors, suggesting that these two carbons arise from an acetate unit. In fact, when [1-^{13}C]acetate was incorporated into the diet, the C-3 signal increased 3% (Table 1) and [1-^{13}C]glucose incorporation enriched C-4 in 1.3% (Table 1). This last result was expected as [1-^{13}C]glucose is first metabolized producing two acetate units, one unlabelled and one labelled at C-2 after a TCA, cycle yielding a weaker enrichment.

This labeling pattern indicates that biosynthesis of **1** occurs via polyketide synthases (PKS) notwithstanding the lack of evidence of their presence in arthropods (see review in ref. 13). On the basis of the PKS mode of action[14] we propose assembling to initiate with a propionyl-CoA (C_3), which is extended by a malonyl-CoA (C_2) and a methylmalonyl-CoA (C_3) (Fig. 3). Hydrolysis of the thioester and decarboxylation yields the saturated ketone precursor of **1** (Fig. 3c). The introduction of the terminal double bond of **1** can be assigned to an unusual desaturase (Fig. 3d), but this mechanism was not assessed in our experiments. Known desaturases in insects do not insert double bonds at the terminal nor at α, β-carbonyl positions[2]. A saturated analog of **1** (4-methyl-3-hexanone) was isolated from the ant *Harpegnathos saltator* and also revealed a mixed acetate-propionate origin by heavy isotope labeling[15]. Among arachnids, the storage mite *Chortoglyphus arcuatus* was shown to adds one acetate and four propionate units to produce the aggregation pheromone (4R,6R,8R)-4,6,8-trimethyldecan-2-one (chortolure)[16].

A closer examination of the ^{13}C NMR spectra from labeling experiments with [4-^{13}C]methylmalonate, [1-^{13}C]acetate and [1-^{13}C]glucose revealed intriguing labeling patterns relative to the C_3 units (Fig. 4). The unexpected labeling of **1** at C-6, by adding [4-^{13}C]methylmalonate to the diet of *Iporangaia* individuals suggests the existence of a methylmalonyl-CoA mutase. The isotopic enhancement at positions C-6 (1.5%) and C-7 (2.3%) complies with the methylmalonyl-CoA–succinyl-CoA interconversion, leading to [2-^{13}C] and [3-^{13}C]propionyl-CoA biosynthesis as consequence of the equilibrium succinyl-CoA-succinate[17] (Fig. 5a1). Stronger enrichment at C-7 indicates the incorporation of [3-^{13}C]propionyl-CoA and the enrichment at

C-1 (1.9%) is provided by direct incorporation of [4-^{13}C]methylmalonyl-CoA as the second extender unit (Fig. 5c). Moreover, labeling of **1** at propionate units with [1-^{13}C]acetate incorporation (Fig. 4) suggests the conversion of acetate into propionate/methylmalonate. The presence of methylmalonyl-CoA would yield [1-^{13}C]methylmalonyl-CoA (Fig. 5c), undetectable due to the loss of labeled carbon, as depicted in Figure 3c. Methylmalonyl-CoA mutase was previously reported in polyketide biosynthesis of insects[18–20] and other arthropods[21], suggesting that **1** from *I. pustulosa* possesses similar biosynthetic origin.

Enrichment at C-5 (1.6%) and C-6 (1.3%) are similar with the [1-^{13}C]acetate feeding, suggesting that both labelings arise from the same pool of starting units, either [1-^{13}C] or [2-^{13}C]propionyl-CoA. The biosynthesis of the C_3 unit from C_2 via α-oxidation and decarboxylation of butyrate has been reported for pheromone biosynthesis of *Carpophilus* beetles (Nitidulidae)[22], however this mechanism is not in accordance with the observed labeling pattern. Chan et al.[23] reviewed the sources of propionate in several microorganisms and, in the crotonyl-CoA carboxylase/reductase pathway, the acetate is converted into propionate after condensation of two acetyl-CoA units yielding a butyrate[24]. The presence of a crotonyl-CoA mutase pathway in *I. pustulosa* would explain [1-^{13}C]acetate labeling of **1** at C-5 and C-6, which corresponds to [1-^{13}C] and [3-^{13}C]propionate incorporation (Fig. 6a). Enrichment at C-1 of **1** by feeding on [1-^{13}C]acetate corresponds to the incorporation of a [3-^{13}C]methylmalonyl-CoA extender unit, whose origin can be linked to methionine and/or threonine catabolism via acetate (Fig. 6c).

[4-^{13}C]methylmalonate incorporation enriches positions C-6 and C-7 of **1**, corresponding to a C_3 starter unit while the C_3 extender unit labels only C-1 and not C-2, (Fig. 5). This labeling pattern indicates the existence of parallel propionate routes in *I. pustulosa* for the C_3 units as starter and extender to be loaded during the polyketide chain assembly. Analyzing [1-^{13}C]acetate incorporation, the labeling at C-6 and non-labeling at C-2 (Fig. 6) are further evidences of these parallel
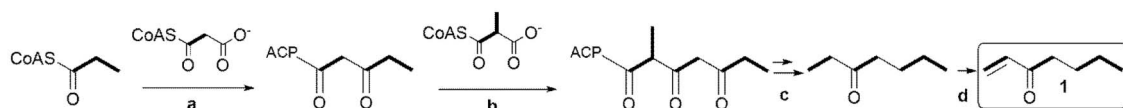

Figure 3 | Proposed biosynthetic pathway for hepten-3-one (1) from the harvestman *Iporangaia pustulosa*. (a) Condensation of propionyl-CoA with malonate. (b) Condensation with methylmalonyl-CoA. (c) Reduction, dehydration, reduction, thioester hydrolysis and decarboxylation. (d) Dehydrogenation of the saturated ketone.
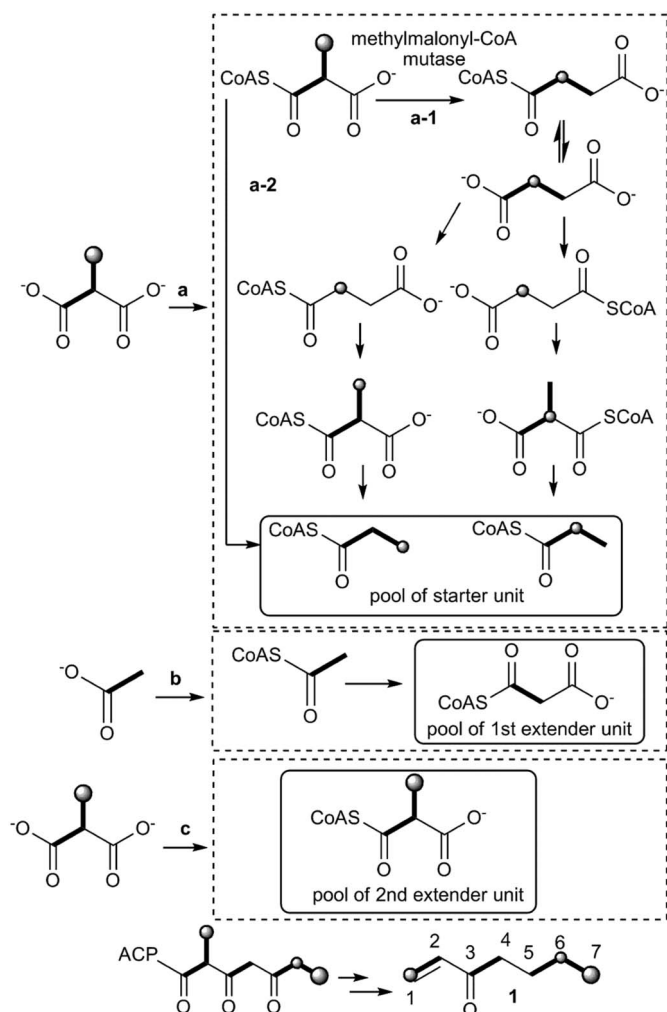
Figure 5 | Proposed biosynthesis of 1 from the harvestman *Iporangaia pustulosa* based on [4-13C]methylmalonate feeding experiment and observed labelling pattern. Isotopomers of 1 and precursor are not indicated separately.

route. Additionally, the [13C₃]propionate 13C enrichment at C-1 and C-2 (15.25 and 16.27%, respectively) corresponding to the extender unit is weaker than at C-5, C-6 and C-7 (39.18%, 48.63% and 45.06%, respectively), the starter unit, which reinforces the hypothesis of parallel propionate route (Table 2).

Feeding on [4-13C]methylmalonate yields no labeling at C-3 nor at C-4 of 1, corresponding to the acetate portion of 1 polyketide chain. Consequently, we can say that harvestman propionate catabolism occurs via succinate, contrary to the alternative biosynthetic pathway via 3-hydroxypropionate to acetate for insects[25,26]. Our results also indicate that propionate in harvestmen may arise from three alternative biosynthetic pathways (succinyl-CoA, crotonyl-CoA and methionine and/or threonine catabolism) (Fig. 7). Bacterial symbionts are known to participate in the biosynthesis of arthropod secondary metabolites[27,28] and may be an alternative source of C₃ units for the biosynthesis of harvestman defense molecules like 1.

Given that the production of vinyl ketones is a synapomorphy of the clade composed of the subfamilies Gonyleptinae, Hernandariinae, Sodreaninae, Progonyleptoidellinae, and Caelopyginae[10], the biosynthetic pathway described here is probably similar in other harvestman species belonging to this major group of gonyleptids. Moreover, given that the production of vinyl ketones is derived from an ancient state of the benzoquinone production in gonyleptid harvestmen[10], our next challenge is to investigate whether distantly
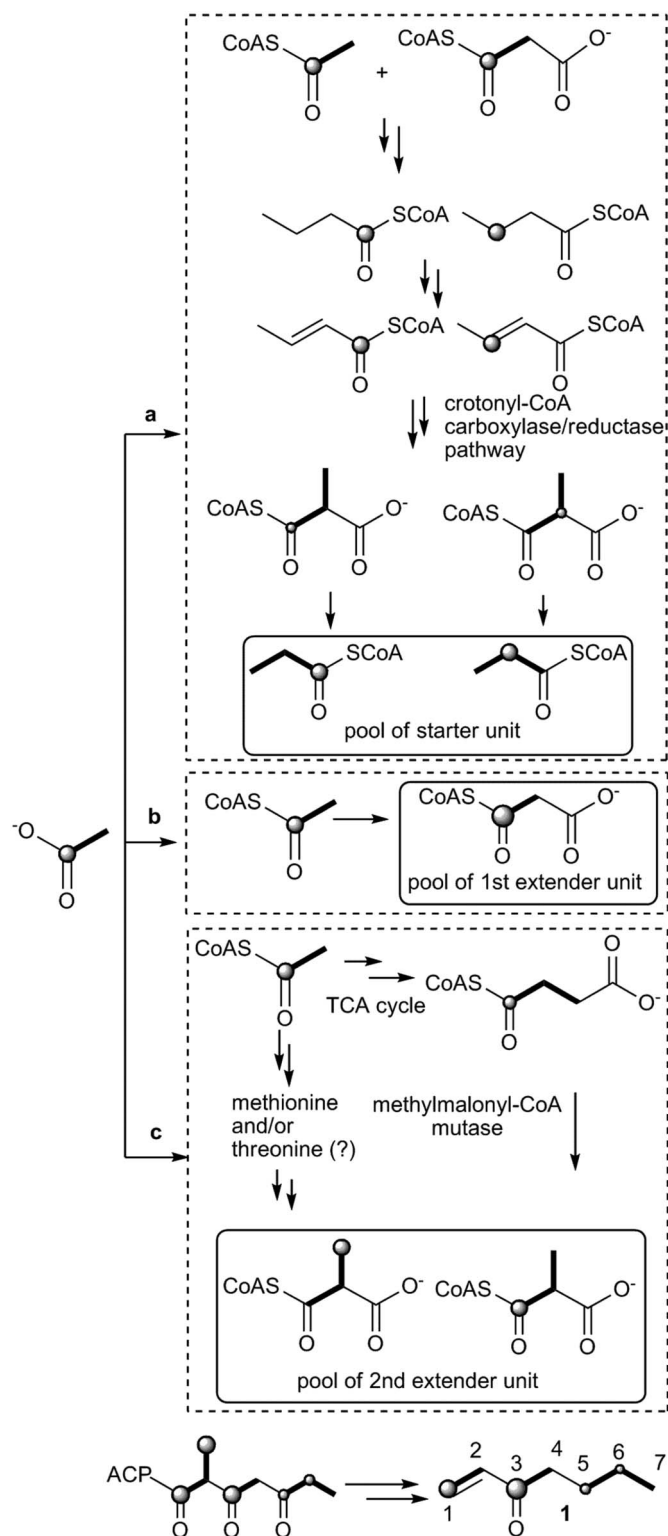

Figure 6 | Proposed biosynthesis of 1 from the harvestman *Iporangaia pustulosa* based on [1-13C]acetate feeding experiment and observed labelling pattern. Isotopomers of 1 and precursors are not indicated separately.

related species partially share the biosynthetic pathways of these two major classes of metabolites. Previous studies with tenebrionid beetles have shown that two 1,4-benzoquinones also reported in the scent gland secretion of several gonyleptid species (2-methyl-1,4-benzoquinone and 2-ethyl-1,4-benzoquinone) are derived from a pathway using acetate and propionate as precursors, suggesting a
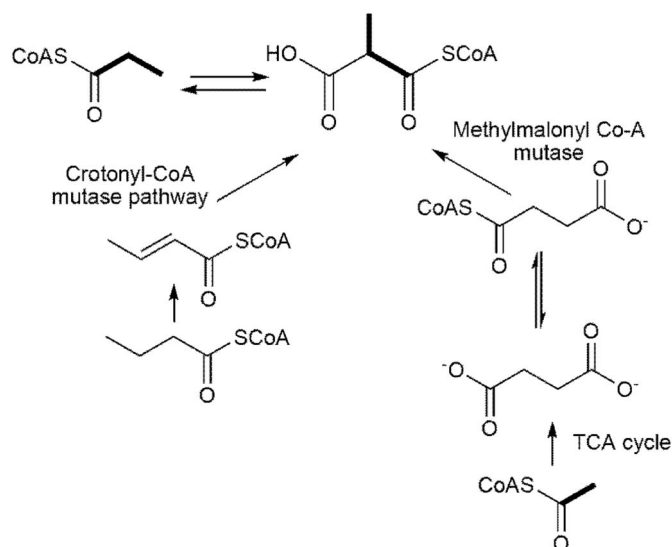
Figure 7 | Propionate metabolism in the harvestman *Iporangaia pustulosa*.

polyketide origin[29,30]. Therefore, similar precursors for both benzo-quinones and vinyl ketones could help to explain evolutionary transitions among gonyleptid species.

Methods

Harvestmen. Individuals of *Iporangaia pustulosa* were collected from a large fragment of Atlantic forest in the state of São Paulo, southeastern Brazil. The collection of the animals in the field and the experiments in the laboratory complied with the current laws of the Brazilian government. Before beginning the experiment, a dorso-ventral pressure was applied on all individuals to empty the gland sacs. The pool of harvestmen was divided into four groups and fed on: 1) canned sardines with 5% w/w [1-^{13}C]sodium acetate (Cambridge Isotope Laboratories, CIL) (n = 14 individuals); 2) canned sardines with 5% w/w [4-^{13}C]sodium methylmalonate (Supplementary Method S7) (n = 11 individuals); 3) canned sardines with 1% w/w [1-^{13}C]glucose (CIL) (n = 10 individuals) and 4) [^{13}C$_3$] propionate (Aldrich) (n = 28 individuals). The control group was the exudate extracted before initiating each experiment (n = 30 individuals). The experiment was set up over a period of 60 days (for groups 1 to 3) and 30 days for group 4, with feeding renewal every 48 h. The number of individuals analyzed in each experimental group is the total that survived for the whole period of feeding (minimum of 10 individuals). The gland exudates were collected with dewaxed cotton wool and extracted from the cotton wool with deuterochloroform or deuterobenzene.

NMR analyses of 1. ^{13}C NMR signal assignment of **1** has been described in the literature[31]. Previous assignments were refined with 2D-NMR spectra (^{1}H, ^{13}C HSQC) (Supplementary Data S9) of the synthetic standard[9]. NMR spectra of **1** were acquired with either a Bruker Avance III 11 tesla (for [1-^{13}C]acetate, [4-13C]methylmalonate and [^{13}C$_3$]propionate groups) or a Varian Inova spectrometer 11 tesla ([1-^{13}C]glucose group), both operating at 125.75 MHz, 25°C, acquisition time 0.55 s, and collecting about 40,000 scans, taking care to have equal scan numbers for samples belonging to the same experiment (sample and control).

1. Eisner, T., Eisner, M. & Seigler, M. *Secret Weapons: Defenses of Insects, Spiders, Scorpions, and Other Many-Legged Creatures,* (Belknap Press of Harvard University Press, 2005).
2. Morgan, E. D. *Biosynthesis in Insects* - Advanced Edition, (RSC, Cambridge 2010).
3. Gnaspini, P. & Hara, M. R. Defense Mechanisms, in: Pinto-da-Rocha, R., Machado, G. & Giribet, G. (Eds.), *Harvestmen: the biology of Opiliones*, 374–399 (Harvard University Press, 2007).
4. Machado, G., Bonato, V. & Oliveira, P. S. Alarm communication: a new function for the scent gland secretion in harvestmen (Arachnida: Opiliones). *Naturwissenschaften*, **89**, 357–360 (2002).
5. Duffield, R. M., Olubajo, O., Wheeler, J. W. & Shear, W. A. Alkylphenols in the defensive secretion of the nearctic opilionid. *Stygnomma spinifera* (Arachnida: Opiliones). *J. Chem. Ecol.* **7**, 445–452 (1981).
6. Eisner, T., Rossini, C., González, A. & Eisner, M. Chemical defense of an opilionid (*Acanthopachylus aculeatus*). *J. Exp. Biol.* **207**, 1313–1321 (2004).
7. Machado, G., Carrera, P. C., Pomini, A. M. & Marsaioli, A. J. Chemical defense in harvestmen (Arachnida, Opiliones): do benzoquinone secretions deter invertebrate and vertebrate predators? *J. Chem. Ecol.* **31**, 2519–2539 (2005).
8. Hara, M. R., Cavalheiro, A. J., Gnaspini, P. & Santos, D. Y. A. C. A comparative analysis of the chemical nature of defensive secretions of Gonyleptidae (Arachnida: Opiliones: Laniatores). *Biochem. Syst. Ecol.* **33**, 1210–1225 (2005).
9. Rocha, D. F. O., Hamilton, K., Gonçalves, C. C. S., Machado, G. & Marsaioli, A. J. 6-Alkyl-3,4-dihydro-2H-pyrans: chemical secretion compounds in neotropical harvestmen. *J. Nat. Prod.* **74**, 658–663 (2011).
10. Caetano, D. S. & Machado, G. The ecological tale of Gonyleptidae (Arachnida, Opiliones) evolution: phylogeny of a Neotropical lineage of armoured harvestmen using ecological, behavioural and chemical characters. *Cladistics* doi:10.1111/cla.12009 (2013).
11. Schneider, B. Nuclear magnetic resonance spectroscopy in biosynthetic studies. *Prog. Nucl. Magn. Reson. Spectrosc.* **51**, 155–198 (2007).
12. Maier, W., Shneider, B. & Strack, D. Biosynthesis of sesquiterpenoid cyclohexenone derivatives in mycorrhizal barley roots proceeds via glyceraldehyde 3-phosphate/pyruvate pathway. *Tetrahedron Lett.* **39**, 521–524 (1998).
13. Pankewitz, F. & Hilker, M. Polyketides in insects: ecological role of these widespread chemicals and evolutionary aspects of their biogenesis. *Biol. Rev.* **83**, 209–226 (2008).
14. Hertweck, C. The biosynthetic logic of polyketide diversity. *Angew. Chem. Int. Ed.* **48**, 4688–4716 (2009).
15. Jarvis, A. P., Liebig, J., Hölldobler, B. & Oldham, N. J. Biosynthesis of the insect pheromone (*S*)-4-methyl-3-heptanone. *Chem. Commun.* 1196–1197 (2004).
16. Schulz, S., Fuhlendorff, J., Steidle, J. L. M., Collatz, J. & Franz, J. T. Identification and biosynthesis of an aggregation pheromone of the storage mite *Chortoglyphus arcuatus*. *ChemBioChem* **5**, 1500–1507 (2004).
17. Amagai, K., Kudo, F. & Eguchi, T. Biosynthetic pathway of macrolactam polyketide antibiotic cremimycin. *Tetrahedron* **67**, 8559–8563 (2011).
18. Halarnkar, P. P. & Blomquist, G. J. Comparative aspects of propionate metabolism. *Comp. Biochem. Physiol.* **92B**, 227–231 (1989).
19. Blomquist, G. J., Halankar, P. P. & Dwyer, L. A. Propionate and methyl malonate metabolism in insects, in: *ACS Symposium Series, Bioregulators for Pest Control*, Vol. **276**, 245–253 (ACS, Washington D.C. (1985).
20. Wakayama, E. D., Dillwith, J. W., Howard, R. W. & Blomquist, G. J. Vitamin B$_{12}$ levels in selected insects. *Insect Biochem.* **14**, 175–179 (1984).
21. Halarnkar, P. P., Chambers, J. D., Wakayama, E. J. & Blomquist, G. J. Vitamin B$_{12}$ levels and propionate metabolism in selected non-insect arthropods and other invertebrates. *Comp. Biochem. Physiol.* **88B**, 869–873 (1987).
22. Petroski, R. J., Bartelt, R. J. & Weisleder, D. Biosynthesis of (2E,4E,6E)-5-Ethyl-3-Methyl-2,4,6-nonatriene: the aggregation pheromone of *Carpophilus freemani* (Coleoptera: Nitidulidae). *Insect Biochem. Mol. Biol.* **24**, 69–78 (1994).
23. Chan, Y. A., Podevels, A. M., Kevany, B. M. & Thomas, M. G. Biosynthesis of polyketide synthase extender units. *Nat. Prod. Rep.* **26**, 90–114 (2009).
24. Erb, T. J. *et al*. Synthesis of C5-dicarboxylic acids from C$_2$-units involving crotonyl-CoA carboxylase/reductase: The ethylmalonyl-CoA pathway. *Proc. Natl. Acad. Sci. U. S. A.* **104**, 10631–10636 (2007).
25. Halarnkar, P. P., Nelson, J. H., Heisler, C. R. & Blomquist, G. J. Metabolism of propionate to acetate in the cockroach *Periplaneta americana*. *Archiv. Biochem. Biophys.* **236**, 526–534 (1985).
26. Halarnkar, P. P., Chambers, J. D. & Blomquist, G. J. Metabolism of propionate to acetate in nine insect species. *Comp. Biochem. Physiol.* **84B**, 469–472 (1986).
27. Crawford, J. M. & Clardy, J. Bacterial symbionts and natural products. *Chem. Commun.* **47**, 7559–7566 (2011).
28. Piel, J. Metabolites from symbiotic bacteria. *Nat. Prod. Rep.* **26**, 338–362 (2009).
29. Meinwald, J., Koch, K. F., Rogers, J. E. Jr. & Eisner, T. Biosynthesis of arthropod secretions. III. Synthesis of simple p-benzoquinones in a beetle (*Eleodes longicollis*). *J. Am. Chem. Soc.* **88**, 1590–1592 (1966).
30. Blum, M. S. Biosynthesis of arthropod exocrine compounds. *Annu. Rev. Entomol.* **32**, 381–413 (1987).
31. Vavrecka von, M. & Hesse, M. Synthesis of monomorin I, a trail pheromone of the pharao ant (*Monomorium pharaonis*). *Helv. Chim. Acta* **74**, 438–444 (1991).

Acknowledgments

Author contributions

D.F.O.R., F.C.W. and A.J.M. are the chemists responsible for the experimental design and data interpretation. G.M. is the biologist responsible for the identification and collection of the individuals and furnished technical support to raise *Iporangaia pustulosa* individuals in the laboratory.

Additional information